

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

| | | |
|---|-----------|---|
| (51) International Patent Classification 6 : A61K 31/40 | A1 | (11) International Publication Number: WO 99/06042 (43) International Publication Date: 11 February 1999 (11.02.99) |
|---|-----------|---|

| | | |
|--|---|--|
| (21) International Application Number: PCT/US98/15909 | (22) International Filing Date: 30 July 1998 (30.07.98) | (81) Designated States: AL, AU, BA, BB, BG, BR, CA, CN, CZ, EE, GE, HU, ID, IL, IS, JP, KP, KR, LC, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, SL, TR, TT, UA, US, UZ, VN, YU, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). |
| (30) Priority Data: 60/054,249 30 July 1997 (30.07.97) US | | Published <i>With international search report.</i> |
| (71) Applicant (<i>for all designated States except US</i>): SMITHKLINE BEECHAM CORPORATION [US/US]; One Franklin Plaza, Philadelphia, PA 19103 (US). | | |
| (72) Inventors; and (75) Inventors/Applicants (<i>for US only</i>): LEE, Dennis [CA/US]; 205 Haverford Avenue, Swarthmore, PA 19081 (US). LONG, Scott, A. [US/US]; 724 Mill Grove Drive, Norristown, PA 19403 (US). | | |
| (74) Agents: VENETIANER, Stephen et al.; SmithKline Beecham Corporation, Corporate Intellectual Property, UW2220, 709 Swedeland Road, P.O. Box 1539, King of Prussia, PA 19406-0939 (US). | | |

(54) Title: CASPASES AND APOPTOSIS

(57) Abstract

The present invention is to the novel compounds of Formula (I), their pharmaceutical compositions, and to the novel inhibition of Caspases for use in the treatment of apoptosis, and disease states caused by excessive or inappropriate cell death.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | | | |
|----|--------------------------|----|---------------------------------------|----|---|----|--------------------------|
| AL | Albania | ES | Spain | LS | Lesotho | SI | Slovenia |
| AM | Armenia | FI | Finland | LT | Lithuania | SK | Slovakia |
| AT | Austria | FR | France | LU | Luxembourg | SN | Senegal |
| AU | Australia | GA | Gabon | LV | Larvia | SZ | Swaziland |
| AZ | Azerbaijan | GB | United Kingdom | MC | Monaco | TD | Chad |
| BA | Bosnia and Herzegovina | GE | Georgia | MD | Republic of Moldova | TG | Togo |
| BB | Barbados | GH | Ghana | MG | Madagascar | TJ | Tajikistan |
| BE | Belgium | GN | Guinea | MK | The former Yugoslav Republic of Macedonia | TM | Turkmenistan |
| BF | Burkina Faso | GR | Greece | ML | Mali | TR | Turkey |
| BG | Bulgaria | HU | Hungary | MN | Mongolia | TT | Trinidad and Tobago |
| BJ | Benin | IE | Ireland | MR | Mauritania | UA | Ukraine |
| BR | Brazil | IL | Israel | MW | Malawi | UG | Uganda |
| BY | Belarus | IS | Iceland | MX | Mexico | US | United States of America |
| CA | Canada | IT | Italy | NE | Niger | UZ | Uzbekistan |
| CF | Central African Republic | JP | Japan | NL | Netherlands | VN | Viet Nam |
| CG | Congo | KE | Kenya | NO | Norway | YU | Yugoslavia |
| CH | Switzerland | KG | Kyrgyzstan | NZ | New Zealand | ZW | Zimbabwe |
| CI | Côte d'Ivoire | KP | Democratic People's Republic of Korea | PL | Poland | | |
| CM | Cameroon | KR | Republic of Korea | PT | Portugal | | |
| CN | China | KZ | Kazakstan | RO | Romania | | |
| CU | Cuba | LC | Saint Lucia | RU | Russian Federation | | |
| CZ | Czech Republic | LI | Liechtenstein | SD | Sudan | | |
| DE | Germany | LK | Sri Lanka | SE | Sweden | | |
| DK | Denmark | LR | Liberia | SG | Singapore | | |
| EE | Estonia | | | | | | |

Caspases and Apoptosis

FIELD OF THE INVENTION

The present invention is to the discovery of a new method to block excessive or
5 inappropriate apoptosis in a mammal.

BACKGROUND

It has been recognized for over a century that there are different forms of cell death. One form of cell death, necrosis, is usually the result of severe trauma and is a
10 process that involves loss of membrane integrity and uncontrolled release of cellular contents, often giving rise to inflammatory responses. In contrast, apoptosis is a more physiological process that occurs in a controlled manner and is generally non-inflamatory in nature. For this reason apoptosis is often referred to as programmed cell death. The name itself (apoptosis: Greek for "dropping off", for example leaves from
15 trees) implies a cell death that is part of a normal physiological process (Kerr et al., Br. J. Cancer, 26: 239-257 (1972)).

Apoptosis appears to be a carefully controlled series of cellular events which ultimately leads to death of the cell. This process for elimination of unwanted cells is active and requires expenditure of cellular energy. The morphological characteristics of
20 apoptosis include cell shrinkage and loss of cell-cell contact, condensation of nuclear chromatin followed by fragmentation, the appearance of membrane ruffling, membrane blebbing and apoptotic bodies. At the end of the process, neighboring cells and macrophages phagocytose the fragments from the apoptotic cell. The process can be very fast, occurring in as little as a few hours (Bright et al., Biosci. Rep., 14: 67-82 (1994)).

25 The best defined biochemical event of apoptosis involves the orderly destruction of nuclear DNA. Signals for apoptosis promote the activation of specific calcium- and magnesium-dependent endonucleases that cleave the double stranded DNA at linker regions between nucleosomes. This results in production of DNA fragments that are multiples of 180-200 base pair fragments (Bergamaschi et al., Haematologica, 79: 86-93
30 (1994); Stewart, JNCI, 86: 1286-1296 (1994)). When examined by agarose gel electrophoresis, these multiple fragments form a ladder pattern that is characteristic for most cells undergoing apoptosis.

There are numerous stimuli that can signal cells to initiate or promote cellular apoptosis, and these can be different in different cells. These stimuli can include
35 glucocorticoids, TNF α , growth factor deprivation, some viral proteins, radiation and anticancer drugs. Some of these stimuli can induce their signals through a variety of cell surface receptors, such as the TNF / nerve growth factor family of receptors, which

include CD40 and Fas/Apo-1 (Bright et al., *supra*). Given this diversity in stimuli that cause apoptosis it has been difficult to map out the signal transduction pathways and molecular factors involved in apoptosis. However, there is evidence for specific molecules being involved in apoptosis.

5 The best evidence for specific molecules that are essential for apoptosis comes from the study of the nematode *C. elegans*. In this system, genes that appear to be required for induction of apoptosis are *Ced-3* and *Ced-4*. These genes must function in the dying cells and, if either gene is inactivated by mutation, cell death fails to occur (Yuan et al., *Devel. Biol.*, 138: 33-41 (1990)). In mammals, genes that have been linked with
10 induction of apoptosis include the proto-oncogene *c-myc* and the tumor suppresser gene *p53* (Bright et al., *supra*; Symonds et al., *Cell*, 78: 703-711 (1994)).

In this critical determination of whether or not to undergo apoptosis, it is not surprising that these are genes that program for proteins that inhibit apoptosis. An example in *C. elegans* is *Ced-9*. When it is abnormally activated, cells survive that would normally die and, conversely, when *Ced-9* is inactivated cells die that would normally live (Stewart, B.W., *supra*). A mammalian counterpart is *bcl-2*, which had been identified as a cancer-causing oncogene. This gene inhibits apoptosis when its product is overexpressed in a variety of mammalian cells, rendering them less sensitive to radiation, cytotoxic drugs and apoptotic signals such as *c-myc* (Bright et al., *supra*). Some virus protein have taken
20 advantage of this ability of specific proteins to block apoptosis by producing homologous viral proteins with analogous functions. An example of such a situation is a protein produced by the Epstein Barr virus that is similar to *bcl-2*, which prevents cell death and thus enhances viral production (Wells et al., *J. Reprod. Fertil.*, 101: 385-391 (1994)). In contrast, some proteins may bind to and inhibit the function of *bcl-2* protein, an example
25 being the protein *bax* (Stewart, B.W., *supra*). The overall picture that has developed is that entry into apoptosis is regulated by a careful balancing act between specific gene products that promote or inhibit apoptosis (Barinaga, *Science*, 263: 754-756 (1994)).

Apoptosis is an important part of normal physiology. The two most often cited examples of this are fetal development and immune cell development. In development of
30 the fetal nervous system, over half of the neurons that exist in the early fetus are lost by apoptosis during development to form the mature brain (Bergamaschi et al., *Haematologica*, 79: 86-93 (1994)). In the production of immune competent T cells (and to a lesser extent evidence exists for B cells), a selection process occurs that eliminates cells that recognize and react against self. This selection process is thought to occur in an
35 apoptotic manner within areas of immune cell maturation (Williams, G. T., *J. Pathol.*, 173: 1-4 (1994); Krammer et al., *Curr. Opin. Immunol.*, 6: 279-289 (1994)).

Dysregulation of apoptosis can play an important role in disease states, and diseases can be caused by both excessive or too little apoptosis occurring. An example of diseases associated with too little apoptosis would be certain cancers. There is a follicular B-cell lymphoma associated with an aberrant expression of functional bcl-2 and an inhibition of apoptosis in that cell (Bergamaschi et al., *supra*). There are numerous reports that associate deletion or mutation of p53 with the inhibition of apoptosis and the production of cancerous cells (Kerr et al., *Cancer*, 73: 2013-2026 (1994); Ashwell et al., *Immunol. Today*, 15: 147-151, (1994)). In contrast, one example of excessive or inappropriate apoptosis is the loss of neuronal cells that occurs in Alzheimer disease, 5 possible induced by b-amyloid peptides (Barr et al., *BioTechnology*, 12: 487-493 (1994)). Other examples include excessive apoptosis of CD4⁺ T cells that occurs in HIV infection, of cardiac myocytes during infarction / reperfusion and of neuronal cells during 10 ischemia (Bergamaschi et al., *supra*); Barr et al., *supra*).

Some pharmacological agents attempt to counteract the lack of apoptosis that is 15 observed in cancers. Examples include topoisomerase II inhibitors, such as the epipodophyllotoxins, and antimetabolites, such as ara-c, which have been reported to enhance apoptosis in cancer cells (Ashwell et al., *supra*). In many cases with these anti-cancer drugs, the exact mechanism for the induction of apoptosis remains to be elucidated.

In the last few years, evidence has built that ICE and proteins homologous to ICE 20 (Caspases) play a key role in apoptosis. This area of research has been spurred by the observation of homology between the protein coded by Ced-3, a gene known to be critical for C. Elegans apoptosis, and ICE (Caspase 1). These two proteins share 29% amino acid identity, and complete identity in the 5 amino acid portion thought to be responsible for protease activity (QACRG) (Yuan et al., *Cell*, 75: 641-652 (1993)). Additional 25 homologies are observed between ICE and the product of the nedd-2 gene in mice, a gene suspected of involvement in apoptosis in the developing brain (Kumar et al., *Genes Dev.*, 8: 1613-1626 (1994)) and Ich-1 (Caspase 2) and CPP32 (Caspase 3), human counterparts of nedd-2 isolated from human brain cDNA libraries (Wang et al., *Cell*, 78: 739-750 (1994); Fernandes-Alnemiri et al., *J. Biol. Chem.*, 269: 30761-30764 (1994)).

30 Further proof for the role of these proteins in apoptosis comes from transfection studies. Over expression of murine ICE caused fibroblasts to undergo programmed cell death in a transient transfection assay (Miura et al., *Cell*, 75: 653-660 (1993)). Cell death could be prevented by point mutations in the transfected gene in the region of greatest homology between ICE and Ced-3. As very strong support for the role of ICE in apoptosis, 35 the authors showed that ICE transfection-induced apoptosis could be antagonized by overexpression of bcl-2, the mammalian oncogene that can prevent programmed cell death (Miura et al., *supra*). Additional experiments were performed using the crmA gene. This

gene of the cowpox virus encodes a serpin protein, a family of proteins that are inhibitors of proteases (Ray et al., Cell, 69: 597-604 (1992)). Specifically, the protein of crmA has been shown to inhibit processing of pro-interleukin -1b by ICE. (Gagliardini et al. Science, 263: 826-828 (1994)) showed that microinjection of the crmA gene into dorsal root
5 ganglion neurons prevented cell death induced by nerve growth factor deprivation. This result shows that ICE is involved in neuronal cell apoptosis. A more direct demonstration of ICE involvement comes from experiments in which ICE transfection is coupled with the co-expression of crmA, demonstrating a crmA-induced suppression of the ICE-induced apoptosis response (Miura et al., *supra*; Wang et al., *supra*).
10 In addition to ICE, researchers have examined the ability of Caspases to promote apoptosis. (Kumar et al. *supra*) demonstrated that over expression of nedd-2 in fibroblasts and neuroblastoma cells resulted in cell death by apoptosis and that this apoptosis could also be suppressed by expression of the bcl-2 gene. Most recently, Wang et al., (Wang et al., *supra*) examined the over expression of Ich-1 in a number of mammalian cells.
15 Expression resulted in cell apoptosis, which could be antagonized by bcl-2 co-expression. Mutation of a cysteine residue, contained within the QACRG motif and presumed to be critical for protease function, to serine abolished apoptotic activity.
Further evidence for a role of a cysteine protease in apoptosis comes from a recent report by Lazebnik et al. (Nature, 371: 346-347 (1994)). These authors have used a cell-
20 free system to mimic and study apoptosis. In their system there is a protease activity that cleaves the enzyme poly(ADP-ribose) polymerase at a site identical to a cleavage site in pre-interleukin-1b. However, this yet to be isolated protease and ICE appear to be different and to act on different substrate proteins. Blockade of protease activity in the system, using non-selective cysteine protease inhibitors, resulted in inhibition of
25 apoptosis.
Taken together, the above evidence provides striking involvement of Caspases in the induction of apoptosis in mammalian cells. Brain interleukin-1 has been reported to be elevated in Alzheimer disease and Down syndrome (Griffin et al., Proc. Natl. Acad. Sci. U. S. A., 86: 7611-7615 (1989)). There are also reports that interleukin-1 can increase the mRNA and production of b-amyloid protein, a major component of senile plaques in Alzheimer disease as well as in brains of people with Down syndrome and with aging (Forloni et al., Mol. Brain Res., 16: 128-134 (1992); Buxbaum et al., Proc. Natl. Acad. Sci. U. S. A., 89: 10075-10078 (1992); Goldgaber et al., Proc. Natl. Acad. Sci. U. S. A., 86: 7606-7610 (1989)). These reports can be viewed as additional evidence for the involvement of ICE in these diseases and the need for use of a novel therapeutic agent and therapy thereby.

To date, no useful therapeutic strategies have blocked excessive or inappropriate apoptosis. In one patent application, EPO 0 533 226 a novel peptide structure is disclosed which is said to be useful for determining the activity of ICE, and therefore useful in the diagnoses and monitoring of IL-1 mediated diseases. Therefore, a need exists to find better therapeutic agents which have non-toxic pharmacological and toxicological profiles for use in mammals. These compounds should block excessive or inappropriate apoptosis cells, and hence provide treatment for diseases and conditions in which this condition appears.

10 SUMMARY OF THE INVENTION

The present invention is to the novel compounds of Formula (I), their pharmaceutical compositions, and to the novel inhibition of Caspases for use in the treatment of apoptosis, and disease states caused by excessive or inappropriate cell death. The compounds of Formula I are most effective in inhibiting Caspases three and seven.

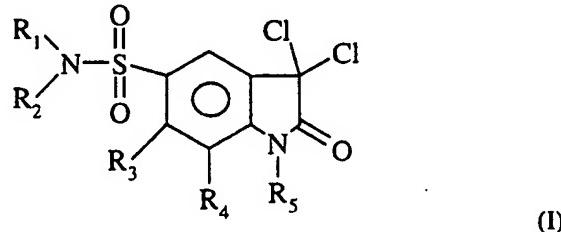
15 Another aspect of the present invention is to a pharmaceutical composition comprising a compound of Formula (I), or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier or diluent.

20 Another aspect of the present invention is to a method for the treatment of diseases or disorders associated with excessive IL-1 β convertase activity, in a mammal in need thereof, which method comprises administering to said mammal an effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

25 Another aspect of the present invention is to a method of preventing or reducing apoptosis in a mammal, preferably a human, in need of such treatment which method comprises administering to said mammal or human an effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Another aspect of the present invention is to a method of blocking or decreasing the production of IL-1 β and/or TNF, in a mammal, preferably a human, in need of such treatment which method comprises administering to said mammal or human an effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

30 The compounds of Formula I are represented by the structure



wherein

R₁ is hydrogen, or C₁₋₄ alkyl;

R₂ is C₁₋₁₀ alkyl, optionally substituted arylC₁₋₄alkyl, optionally substituted heteroaryl

C₁₋₄ alkyl, optionally substituted C₃₋₇ cycloalkyl, or R₁ and R₂ together with the

5 nitrogen to which they are attached from a 3 to 10 membered ring which optionally contains an additional heteroatom selected from oxygen, nitrogen or sulfur;

R₃ and R₄ are C₁₋₆alkyl, hydrogen, nitro, or halogen and

R₅ is C₁₋₆alkyl, hydrogen, arylalkyl or heteroarylkyl.

10 Preferably R₁ and R₂ are joined to form a five membered nitrogen containing ring. It is recognized that the alkyl group in the arylalkyl or heteroalkyl moiety may be branched or straight, such as a methylene or a substituted methylene group, i.e., -CH(CH₃) - aryl. The optionally substituted aryl moiety of the arylalkyl group, may be substituted one to three times independently by hydroxy, halogen, alkyl or alkoxy. R₅ is
15 preferably benzyl.

Compounds exemplified by Formula (I) include, but are not limited to:

5-Chlorosulfonyl-3,3-dichloro-2-oxindole

20 5-Benzylaminosulfonyl-3,3-dichloro-2-oxindole

5-[N-(1-Methyl-3-phenylpropylamino)sulfonyl]-3,3-dichloro-2-oxindole

5-[N-(N-Benzyl-2-cyanoethylamino)sulfonyl]-3,3-dichloro-2-oxindole

5-[N-(2-(3-Pyridyl)ethylamino)sulfonyl]-3,3-dichloro-2-oxindole

5-[N-(2-Furfurylamino)sulfonyl]-3,3-dichloro-2-oxindole

25 5-[N-(2-Isopropoxyethylamino)sulfonyl]-3,3-dichloro-2-oxindole

5-[N-(2-Methoxyethylamino)sulfonyl]-3,3-dichloro-2-oxindole

5-[N-(2-Tetrahydrofurfurylamino)sulfonyl]-3,3-dichloro-2-oxindole

(-)-5-[N-(cis-Myrtanylarnino)sulfonyl]-3,3-dichloro-2-oxindole

5-[(1-Benzylpiperidinyl-4-amino)sulfonyl]-3,3-dichloro-2-oxindole

30 5-[N-(2-Indanamino)sulfonyl]-3,3-dichloro-2-oxindole

5-[N-(Cyclopropylmethylamino)sulfonyl]-3,3-dichloro-2-oxindole

5-[N-(1,5-Dimethylhexylamino)sulfonyl]-3,3-dichloro-2-oxindole

5-[N-(N-Methylbenzylamino)sulfonyl]-3,3-dichloro-2-oxindole

(+/-)-5-[N-(3-(N-Acetyl-N-methylamino)pyrrolidinyl)sulfonyl]-3,3-dichloro-2-oxindole

35 5-[2-(1,2,3,4-Tetrahydroisoquinolino)sulfonyl]-3,3-dichloro-2-oxindole

(+/-)-5-[1-(Decahydroisoquinolino)sulfonyl]-3,3-dichloro-2-oxindole

5-[N-(N-Methyl-2-cyanoethylamino)sulfonyl]-3,3-dichloro-2-oxindole

- 5-[N-(N-Methylcyanomethylamino)sulfonyl]-3,3-dichloro-2-oxindole
- 5-[N-(Pyrrolidinyl)sulfonyl]-3,3-dichloro-2-oxindole
- 5-[N-(N-Methylphenethylamino)sulfonyl]-3,3-dichloro-2-oxindole
- 5-[N-(Azacyclooctane)sulfonyl]-3,3-dichloro-2-oxindole
- 5-[N-(3-Azabicyclo[3.2.2]nonane)sulfonyl]-3,3-dichloro-2-oxindole
- 5-[1-(2-Ethoxycarbonylpiperidinyl)sulfonyl]-3,3-dichloro-2-oxindole
- 5-[N-(Morpholino)sulfonyl]-3,3-dichloro-2-oxindole
- (S)-(+)-5-[1-(2-Methoxymethylpyrrolidinyl)sulfonyl]-3,3-dichloro-2-oxindole
- 5-[N-(N-Methyl-2-(4-pyridinyl)ethylamino)sulfonyl]-3,3-dichloro-2-oxindole
- 10 5-[N-(N-Methyl-2-hydroxyethylamino)sulfonyl]-3,3-dichloro-2-oxindole
- (S)-(+)-5-[N-(2-Hydroxymethylpyrrolidinyl)sulfonyl]-3,3-dichloro-2-oxindole
- (+/-)-5-[1-(3-Hydroxypyrrrolidinyl)sulfonyl]-3,3-dichloro-2-oxindole
- (+/-)-5-[1-(3-aminocarbonylpiperidinyl)sulfonyl]-3,3-dichloro-2-oxindole
- (+/-)-5-[1-(2-Methylpiperidinyl)sulfonyl]-3,3-dichloro-2-oxindole
- 15 15 5-[1-(4-Methylpiperidinyl)sulfonyl]-3,3-dichloro-2-oxindole
- 5-[1-(4-Hydroxypiperidinyl)sulfonyl]-3,3-dichloro-2-oxindole
- (+/-)-5-[1-(2-Hydroxyethyl)piperidinyl)sulfonyl]-3,3-dichloro-2-oxindole
- (+/-)-5-[1-(3-Hydroxymethylpiperidinyl)sulfonyl]-3,3-dichloro-2-oxindole
- 5-[1-(4-Phenylpiperidinyl)sulfonyl]-3,3-dichloro-2-oxindole
- 20 5-[1-(4-Benzylpiperidinyl)sulfonyl]-3,3-dichloro-2-oxindole
- 5-[1-(4-(1-Piperidinyl)piperidinyl)sulfonyl]-3,3-dichloro-2-oxindole
- 5-Benzylaminosulfonyl-N-methyl-3,3-dichloro-2-oxindole
- 5-Chlorosulfonyl-N-methyl-3,3-dichloro-2-oxindole
- 5-Benzylaminosulfonyl-N-methyl-3,3-dichloro-2-oxindole
- 25 N-Methyl-5-(1-piperidinylsulfonyl)-3,3-dichloro-2-oxindole

The term "excessive IL-1b convertase activity" is used herein to mean an excessive expression of the protein, or activation of the enzyme.

The term "C₁₋₆ alkyl" or "alkyl" is used herein to mean both straight and branched chain radicals of 1 to 6 carbon atoms, unless the chain length is otherwise specified, including, but not limited to, methyl, ethyl, *n*-propyl, *iso*-propyl, *n*-butyl, *sec*-butyl, *iso*-butyl, *tert*-butyl, and the like.

The term "heteroaryl" (on its own or in any combination, such as "heteroaryloxy", or "heteroaryl alkyl") is used herein to mean a 5-10 membered aromatic ring system in which one or more rings contain one or more heteroatoms selected from the group consisting of N, O or S, such as, but not limited, to pyrrole, pyrazole, furan, thiophene, quinoline, isoquinoline,

quinazolinyl, pyridine, pyrimidine, oxazole, oxadiazole, tetrazole, thiazole, thiadiazole, triazole, imidazole, benzimidazole, benzothiaphene, benzopyrrole, or benzofuran.

The term "aryl" (on its own or in any combination, such as "aryloxy", or "arylalkyl") is used herein to mean a phenyl and naphthyl ring.

5 The term "cycloalkyl" is used herein to mean cyclic radicals, preferably of 3 to 7 carbons, including but not limited to cyclopropyl, cyclopentyl, cyclohexyl, and the like.

The term "halo" or "halogens", is used herein to include, unless otherwise specified, chloro, fluoro, bromo and iodo.

10 The present invention contains the inhibition of Caspases by compounds of Formula (I). What is meant by the term "Caspases" are fragment, homologs, analogs and derivatives of the polypeptides Interleukin-1 b converting enzyme (or convertase). These analogs are structurally related to the Caspase family. They generally encode a protein (s) which exhibits high homology to the human ICE over the entire sequence. Preferably, the pentapeptide

15 QACRG is conserved. The Caspases , which may include many natural allelic variants (such as substitutions, deletion or addition of nucleotides) does not substantially alter the function of the encoded polypeptide. That is they retain essentially the same biological function or activity as the ICE protease, although it is recognized that the biological function may be enhanced or reduced activity. The suitable activity is not IL-1b convertase activity, but the ability to induce 20 apoptosis or involved in programmed cell death in some manner. Suitable Caspases encompasses within this invention are those described in PCT US94/07127 filed 23 June 1994, Attorney Docket No.: 325800-184; and in USSN 08/334,251, filed 1 November 1994, Attorney Docket No.: 325800-249 whose disclosures are incorporated herein by reference in their entirety.

25 The term "blocking or inhibiting, or decreasing the production of IL-1b and/or TNF" as used herein refers to:

a) a decrease of excessive levels, or a down regulation, of the cytokine in a human to normal or sub-normal levels by inhibition of the *in vivo* release of the cytokine; or

b) a down regulation, at the genomic level, of excessive *in vivo* levels of the cytokine

30 (IL-1 or TNF) in a human to normal or sub-normal levels; or

c) a down regulation, by inhibition of the direct synthesis of the cytokine (IL-1, or TNF) as a posttranslational event; or

d) a down regulation, at the translational level, of excessive *in vivo* levels of the cytokine (IL-1, or TNF) in a human to normal or sub-normal levels.

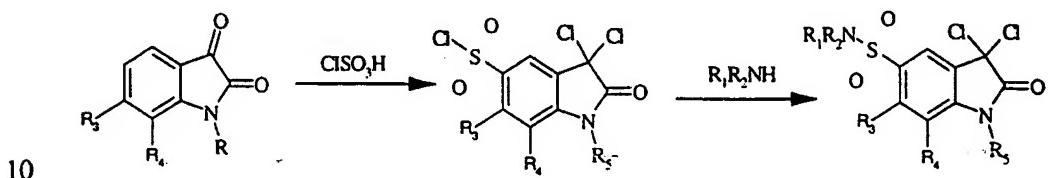
35

The blocking or inhibiting, or decreasing the production of IL-1b and/or TNF is a discovery that the compounds of Formula (I) are inhibitors of the cytokines, IL-1 and TNF is

based upon the effects of the compounds of Formulas (I) on the production of the IL-1 and TNF in *in vitro* and *in vivo* assays which are well known and recognized in the art, some of which are described herein.

- 5 Compound of the present invention may be synthesized in accordance with the schemes illustrated below.

5-Alkylaminosulfonyl-3,3-dichloro-2-oxindoles



- Isatin or its N-alkyl derivative is treated with chlorosulfonic acid at temperatures ranging from 0-10°C in order to obtain 5-chlorosulfonyl-3,3-dichloro-2-oxindole, the direct precursor to the compounds of this invention. Treatment of the chlorosulfonyl derivative with a primary or secondary amine in organic solvents such as tetrahydrofuran, methylene chloride or dimethylformamide with or without the addition of a tertiary amine base such as triethylamine yields the 5-alkylaminosulfonyl-3,3-dichloro-2-oxindole.
- 15

Example 1 (SB263831)

a) 5-Chlorosulfonyl-3,3-dichloro-2-oxindole

- 20 A solution of isatin (1.6 g, 10 mmol) in chlorosulfonic acid (6.6 mL) was heated to 70°C for 3 h. After cooling to RT, the solution was poured into ice and extracted with methylene chloride. The organic solution was dried over magnesium sulfate, filtered, and the solvent was removed under reduced pressure to afford the title compound as an orange solid in quantitative yield. ^1H NMR (400 MHz, CDCl_3) δ 7.25 (d, $J=10.5$ Hz, 1H), 8.12 (d, $J=10.5$ Hz, 1H), 8.29 (s, 1H), 8.74 (br s, 1H).
- 25

b) 5-Benzylaminosulfonyl-3,3-dichloro-2-oxindole

- To a solution of 5-chlorosulfonyl-3,3-dichloro-2-oxindole (100 mg, 400 umol) in methylene chloride (3 mL) was added benzylamine (135 uL, 1.2 mmol) dropwise. After stirring for 4 h. 3 N hydrochloric acid was added along with an additional volume of 30 methylene chloride (20 mL). The organic layer was dried over magnesium sulfate, filtered, and silica gel flash chromatography (25 to 35% ethyl acetate/hexanes) yielded the title compound. ES (-) MS m/e = 369 (M-H).

Example 2 (SB264862)5-[N-(1-Methyl-3-phenylpropylamino)sulfonyl]-3,3-dichloro-2-oxindole

Prepared according to the procedure of example 1b) except substituting 1-methyl-3-phenylpropylamine for benzylamine afforded the title compound as a yellow foam in 5 32% yield. ES (+) MS m/e = 413 (M+H).

Example 3 (SB264860)5-[N-(N-Benzyl-2-cyanoethylamino)sulfonyl]-3,3-dichloro-2-oxindole

Prepared according to the procedure of example 1b) except substituting N-(2-cyanoethyl)benzylamine for benzylamine afforded the title compound as a white solid in 10 19% yield. ES (+) MS m/e = 424 (M-H).

Example 4 (SB265240)5-[N-(2-(3-Pyridyl)ethylamino)sulfonyl]-3,3-dichloro-2-oxindole

15 Prepared according to the procedure of example 1b) except substituting 2-(3-pyridyl)ethylamine for benzylamine afforded the title compound as a yellow oil in 4% yield. ES (+) MS m/e = 386 (M+H).

Example 5 (SB265241)20 5-[N-(2-Furfurylamino)sulfonyl]-3,3-dichloro-2-oxindole

Prepared according to the procedure of example 1b) except substituting 2-furfurylamine for benzylamine afforded the title compound as a yellow solid in 17% yield. ES (-) MS m/e = 359 (M-H).

25 Example 6 (SB265242)5-[N-(2-Isopropoxyethylamino)sulfonyl]-3,3-dichloro-2-oxindole

Prepared according to the procedure of example 1b) except substituting 2-isopropoxyethylamine for benzylamine afforded the title compound as a yellow foam in 33% yield. ES (-) MS m/e = 365 (M-H).

30 Example 7 (SB265243)5-[N-(2-Methoxyethylamino)sulfonyl]-3,3-dichloro-2-oxindole

Prepared according to the procedure of example 1b) except substituting 2-methoxyethylamine for benzylamine afforded the title compound as a yellow foam in 27% 35 yield. ES (-) MS m/e = 337 (M-H).

Example 8 (SB265244)5-[N-(2-Tetrahydrofuryl amino)sulfonyl]-3,3-dichloro-2-oxindole

Prepared according to the procedure of example 1b) except substituting 2-tetrahydrofurylamine for benzylamine afforded the title compound as a light yellow foam in 30% yield. ES (-) MS m/e = 363 (M-H).

Example 9 (SB265246)(-)-5-[N-(cis-Myrtanyl amino)sulfonyl]-3,3-dichloro-2-oxindole

Prepared according to the procedure of example 1b) except substituting (-)-cis-myrtanylamine for benzylamine afforded the title compound as a light yellow foam in 8% yield. ES (-) MS m/e = 415 (M-H).

Example 10 (SB265247)5-[(1-Benzylpiperidinyl-4-amino)aminosulfonyl]-3,3-dichloro-2-oxindole

Prepared according to the procedure of example 1b) except substituting 4-amino-1-benzylpiperidine for benzylamine afforded the title compound as a yellow oil in 5% yield. ES (-) MS m/e = 452 (M-H).

Example 11 (SB265248)5-[N-(2-Indanamino)sulfonyl]-3,3-dichloro-2-oxindole

Prepared according to the procedure of example 1b) except substituting 2-indanamine for benzylamine afforded the title compound as a brown foam in 50% yield. ES (-) MS m/e = 395 (M-H).

Example 12 (SB265249)5-[N-(Cyclopropylmethyl amino)sulfonyl]-3,3-dichloro-2-oxindole

Prepared according to the procedure of example 1b) except substituting cyclopropylmethylamine for benzylamine afforded the title compound as a yellow foam in 21% yield. ES (-) MS m/e = 333 (M-H).

30

Example 13 (SB265250)5-[N-(1,5-Dimethylhexyl amino)sulfonyl]-3,3-dichloro-2-oxindole

Prepared according to the procedure of example 1b) except substituting 1,5-dimethylhexylamine for benzylamine afforded the title compound as a yellow foam in 12% yield. ES (-) MS m/e = 391 (M-H).

Example 14 (SB265550)5-[N-(N-Methylbenzylamino)sulfonyl]-3,3-dichloro-2-oxindole

Prepared according to the procedure of example 1b) except substituting N-methylbenzylamine for benzylamine afforded the title compound as a yellow solid in 38% yield. ES (-) MS m/e = 384 (M-H).

Example 15 (SB265551)(+/-)-5-[N-(3-(N-Acetyl-N-methylamino)pyrrolidinyl)sulfonyl]-3,3-dichloro-2-oxindole

Prepared according to the procedure of example 1b) except substituting 3-(N-acetyl-N-methylamino)pyrrolidine for benzylamine afforded the title compound as a yellow foam in 39% yield. ES (-) MS m/e = 404 (M-H).

Example 16 (SB265594)5-[2-(1,2,3,4-Tetrahydroisoquinolino)sulfonyl]-3,3-dichloro-2-oxindole

Prepared according to the procedure of example 1b) except substituting 1,2,3,4-tetrahydroisoquinoline for benzylamine afforded the title compound as a yellow solid in 47% yield. ES (-) MS m/e = 395 (M-H).

Example 17 (SB265595)(+/-)-5-[1-(Decahydroisoquinolino)sulfonyl]-3,3-dichloro-2-oxindole

Prepared according to the procedure of example 1b) except substituting decahydroisoquinoline for benzylamine afforded the title compound as a yellow foam in 27% yield. ES (-) MS m/e = 401 (M-H).

Example 18 (SB265596)5-[N-(N-Methyl-2-cyanoethylamino)sulfonyl]-3,3-dichloro-2-oxindole

Prepared according to the procedure of example 1b) except substituting N-methyl-beta-alanenitrile for benzylamine afforded the title compound as a light yellow solid in 28% yield. ES (-) MS m/e = 346 (M-H).

30

Example 19 (SB265597)5-[N-(N-Methylcyanomethylamino)sulfonyl]-3,3-dichloro-2-oxindole

Prepared according to the procedure of example 1b) except substituting N-methylaminoacetonitrile for benzylamine afforded the title compound as a yellow solid in 7% yield. ES (-) MS m/e = 332 (M-H).

Example 20 (SB265598)5-[N-(Pyrrolidinyl)sulfonyl]-3,3-dichloro-2-oxindole

Prepared according to the procedure of example 1b) except substituting pyrrolidine for benzylamine afforded the title compound as a light yellow solid in 21% yield. ES (-) MS m/e = 333 (M-H).

Example 21 (SB265599)5-[N-(N-Methylphenethylamino)sulfonyl]-3,3-dichloro-2-oxindole

Prepared according to the procedure of example 1b) except substituting N-Methylphenethylamine for benzylamine afforded the title compound as a light yellow foam in 45% yield. ES (-) MS m/e = 397 (M-H).

Example 22 (SB265600)5-[N-(Azacyclooctane)sulfonyl]-3,3-dichloro-2-oxindole

Prepared according to the procedure of example 1b) except substituting azacyclooctane for benzylamine afforded the title compound as a yellow foam in 49% yield. ES (-) MS m/e = 375 (M-H).

Example 23 (SB265601)5-[N-(3-Azabicyclo[3.2.2]nonane)sulfonyl]-3,3-dichloro-2-oxindole

Prepared according to the procedure of example 1b) except substituting 3-azabicyclo[3.2.2]nonane for benzylamine afforded the title compound as a yellow foam in 60% yield. ES (-) MS m/e = 387 (M-H).

Example 24 (SB265602)5-[1-(2-Ethoxycarbonylpiperidinyl)sulfonyl]-3,3-dichloro-2-oxindole

Prepared according to the procedure of example 1b) except substituting ethyl piperidine-2-carboxylate for benzylamine afforded the title compound as a light yellow foam in 69% yield. ES (-) MS m/e = 419 (M-H).

30

Example 25 (SB265603)5-[N-(Morpholino)sulfonyl]-3,3-dichloro-2-oxindole

Prepared according to the procedure of example 1b) except substituting morpholine for benzylamine afforded the title compound as a light yellow solid in 56% yield. ES (-) MS m/e = 349 (M-H).

Example 26 (SB265604)(S)-(+)-5-[1-(2-Methoxymethylpyrrolidinyl)sulfonyl]-3,3-dichloro-2-oxindole

Prepared according to the procedure of example 1b) except substituting (S)-(+)-2-(methoxymethyl)pyrrolidine for benzylamine afforded the title compound as a light yellow foam in 38% yield. ES (-) MS m/e = 377 (M-H).

Example 27 (SB265605)5-[N-(N-Methyl-2-(4-pyridine)ethylamino)sulfonyl]-3,3-dichloro-2-oxindole

Prepared according to the procedure of example 1b) except substituting N-methyl-2-(4-pyridine)ethylamine for benzylamine afforded the title compound as a brown solid in 38% yield. ES (-) MS m/e = 398 (M-H).

Example 28 (SB265606)5-[N-(N-Methyl-2-hydroxyethylamino)sulfonyl]-3,3-dichloro-2-oxindole

Prepared according to the procedure of example 1b) except substituting N-(methyl)aminoethanol for benzylamine afforded the title compound as a light yellow foam in 27% yield. ES (-) MS m/e = 337 (M-H).

Example 29 (SB265607)(S)-(+)-5-[N-(2-Hydroxymethylpyrrolidinyl)sulfonyl]-3,3-dichloro-2-oxindole

Prepared according to the procedure of example 1b) except substituting (S)-(+)-2-(hydroxymethyl)pyrrolidine for benzylamine afforded the title compound as a light yellow foam in 31% yield. ES (-) MS m/e = 363 (M-H).

25

Example 30 (SB265608)(+/-)-5-[1-(3-Hydroxypyrrolidinyl)sulfonyl]-3,3-dichloro-2-oxindole

Prepared according to the procedure of example 1b) except substituting 3-hydroxypyrrolidine for benzylamine afforded the title compound as a white solid in 9% yield. ES (+) MS m/e = 351 (M+H).

30

Example 31 (SB265609)(+/-)-5-[1-(3-aminocarbonylpiperidinyl)sulfonyl]-3,3-dichloro-2-oxindole

Prepared according to the procedure of example 1b) except substituting 3-carboxamidepiperidine for benzylamine afforded the title compound as a white solid in 53% yield. ES (-) MS m/e = 390 (M-H).

Example 32 (SB266638)(+/-)-5-[1-(2-Methylpiperidinyl)sulfonyl]-3,3-dichloro-2-oxindole

Prepared according to the procedure of example 1b) except substituting 2-methylpiperidine for benzylamine afforded the title compound as a white foam in 10% yield. ES (-) MS m/e = 361 (M-H).

Example 33 (SB266639)(+/-)-5-[1-(4-Methylpiperidinyl)sulfonyl]-3,3-dichloro-2-oxindole

Prepared according to the procedure of example 1b) except substituting 4-methylpiperidine for benzylamine afforded the title compound as an off white solid in 32% yield. ES (-) MS m/e = 361 (M-H).

Example 34 (SB264732)5-[1-(4-Hydroxypiperidinyl)sulfonyl]-3,3-dichloro-2-oxindole

Prepared according to the procedure of example 1b) except substituting 4-hydroxypiperidine for benzylamine afforded the title compound as an off white solid. ES (+) MS m/e = 365 (M+H).

Example 35 (SB264733)(+/-)-5-[1-(2-(2-Hydroxyethyl)piperidinyl)sulfonyl]-3,3-dichloro-2-oxindole

Prepared according to the procedure of example 1b) except substituting (+/-)-2-(2-hydroxyethyl)piperidine for benzylamine afforded the title compound as an off white solid. ES (-) MS m/e = 391 (M-H).

Example 36 (SB264734)(+/-)-5-[1-(3-Hydroxymethylpiperidinyl)sulfonyl]-3,3-dichloro-2-oxindole

Prepared according to the procedure of example 1b) except substituting (+/-)-3-hydroxymethylpiperidine for benzylamine afforded the title compound as an off white solid. ES (-) MS m/e = 377 (M-H).

30

Example 37 (SB264735)5-[1-(4-Phenylpiperidinyl)sulfonyl]-3,3-dichloro-2-oxindole

Prepared according to the procedure of example 1b) except substituting (+/-)-3-hydroxymethylpiperidine for benzylamine afforded the title compound as an off white solid. ES (-) MS m/e = 423 (M-H).

Example 38 (SB264736)5-[1-(4-Benzylpiperidinyl)sulfonyl]-3,3-dichloro-2-oxindole

Prepared according to the procedure of example 1b) except substituting 4-benzylpiperidine for benzylamine afforded the title compound as an off white solid. ES (-) MS m/e = 437 (M-H).

Example 39 (SB264863)5-[1-(4-(1-Piperidinyl)piperidinyl)sulfonyl]-3,3-dichloro-2-oxindole

Prepared according to the procedure of example 1b) except substituting 4-(1-piperidinyl)piperidine for benzylamine afforded the title compound as an off white solid. ES (-) MS m/e = 430 (M-H).

Example 40 (SB263985)5-Benzylaminosulfonyl-N-methyl-3,3-dichloro-2-oxindole

15 a) 5-Chlorosulfonyl-N-methyl-3,3-dichloro-2-oxindole

The title compound was prepared according to the procedure of 1a) except substituting N-methylisatin for isatin. The product was obtained as an orange solid in quantitative yield. ^1H NMR (400 MHz, CDCl_3) d3.37 (s, 3H), 7.09 (d, J=10.5 Hz, 1H), 8.16 (d, J=10.5 Hz, 1H), 8.28 (s, 1H).

20

b) 5-Benzylaminosulfonyl-N-methyl-3,3-dichloro-2-oxindole

To a solution of 5-chlorosulfonyl-N-methyl-3,3-dichloro-2-oxindole (120 mg, 382 umol) in methylene chloride (5 mL) was added benzyl amine (50 uL, 458 umol) dropwise. After stirring overnight, 3 N hydrochloric acid was added along with an additional volume of methylene chloride (20 mL). The organic layer was dried over magnesium sulfate, filtered, and silica gel flash chromatography (35 to 55% ethyl acetate/hexanes) yielded the title compound. ES (-) MS m/e = 383 (M-H).

Example 41 (SB263921)

30 N-Methyl-5-(1-piperidinylsulfonyl)-3,3-dichloro-2-oxindole

Prepared according to the procedure of example 41b) except substituting piperidine for benzylamine. ^1H NMR (400 MHz, CDCl_3) d1.45 (m, 2H), 1.67 (m, 4H), 3.00 (m, 4H), 3.36 (s, 3H), 7.01 (d, J=10.5 Hz, 1H), 7.84 (d, J=10.5 Hz, 1H), 7.99 (s, 1H).

35 Preparation of Active Caspase 3

Full length Caspase 3 was expressed intracellularly in E.coli with N-terminal hexa His tag. E coli cells were lysed in 10 ml/g of cells of lysis buffer (50 mM Na phosphate pH 7.2, 0.1

- M NaCl, 0.1 % Tween 20, and 10 mM b-mercaptoethanol) using Microfluidics M110Y homogenizer at 10,000 psi. After centrifugation, Caspase 3 activity was detected in lysate supernatant. The supernatant was buffer-exchanged on Sephadex G25 column equilibrated with 20 mM TrisHCl, 10 % Sucrose, 0.1 % CHAPS, 2 mM DTT, pH 7.8 (TSCD).
- 5 Fractions containing Caspase 3 activity was applied to DEAE Toyopearl 650 M (Supelco Inc) equilibrated with Buffer TSCD. The column was eluted with a linear gradient of 20 mM to 120 mM of Tris Hcl pH 7.8 in TSCD. Caspase 3 was eluted in early of the gradient before the majority of impurities eluted. This partially purified Capase 3 was used for inhibitor screening. All operations were performed at 4°C and Caspase activity was
- 10 measured using substrate, DEVD-AMC , and Dynatach Fluolite 1000 plate reader.

Caspase 3 Inhibition Assay

- Caspase 3 was assayed at 30 degrees C in 96-well plates using the fluorogenic tetrapeptide substrate *N*-acetyl-L-aspartyl-L-glutamyl-L-valyl-L-aspartyl-7-amido-4-methylcoumarin (Ac-DEVD-AMC). The assays were conducted at pH 7.5 in a buffered system containing 25 mM Hepes, 10% sucrose, 0.1% CHAPS, and 1-50 uM DTT. The concentration of substrate was fixed at 10 uM. Fluorescence of the liberated 7-amino-4-methylcoumarin was continuously monitored at 460 nm following excitation at 360 nm.

20 ***Compound Testing***

Compounds were tested at a single dose of 50 to 100 uM. Activity was monitored as described above over a 30 to 60-minute time period following the simultaneous addition of substrate and inhibitor to enzyme to initiate the reaction. The progress curves thus generated were fit by computer to Eq. 1 in order to assess potency and/or time-dependency:

25

$$v = \frac{(V_o(1 - e^{-k_{obs}t})}{k_{obs}} \quad (1)$$

Representative compounds of formula (I) have demonstrated positive inhibitory activity in the above noted assay.

30

Apoptosis Assay (Jurkat Cells):

Materials : Compounds

- Compounds were made as stocks (5-100 mM) in dimethylsulfoxide (DMSO) and diluted in DMSO to provide final concentrations, with DMSO concentrations ranging from 35 0.1-1%.

Preparation of cells

5 Jurkat cells were obtained from American Type Culture Collection and grown in RPMI-1640 media supplemented with 10% fetal bovine serum at 37°, 5% CO₂. Cells were seeded in T-flasks at 0.03 to 0.08 x 10⁶ cells / ml and used for experiments at 0.5 to 1.0 x 10⁶ cells / ml. Other proliferative cells can be used with apoptosis induced by anti-fas, camptothecine, ceramide or TNF.

Apoptosis Assay

10 A method for measuring apoptosis is to quantitate the amount of broken DNA fragments using a fluorescent end-labeling method, a system used in the ApopTag kit from Oncor (Gaithersburg, MD). In brief, the enzyme terminal deoxynucleotidyl transferase extends the DNA fragments with digoxigenin-containing nucleotides, which are then detected with an antidigoxigenin antibody carrying fluorescein to allow detection by fluorescence (494 nm excitation and 523 nm emission). Propidium iodide is used as counter stain to measure total DNA content. Flow cytometric analysis was done on Becton-Dickinson (Rutherford, NJ) FACScan instrument using CellQuest software.

METHODS OF TREATMENT

20 For therapeutic use the compounds of the present invention will generally be administered in a standard pharmaceutical composition obtained by admixture with a pharmaceutical carrier or diluent selected with regard to the intended route of administration and standard pharmaceutical practice. For example, they may be administered orally in the form of tablets containing such excipients as starch or lactose, or in capsule, ovules or
25 lozenges either alone or in admixture with excipients, or in the form of elixirs or suspensions containing flavouring or colouring agents. They may be injected parenterally, for example, intravenously, intramuscularly or subcutaneously. For parenteral administration, they are best used in the form of a sterile aqueous solution which may contain other substances, for example, enough salts or glucose to make the solution isotonic with blood. The choice of
30 form for administration as well as effective dosages will vary depending, inter alia, on the condition being treated. The choice of mode of administration and dosage is within the skill of the art.

The compounds of the present invention, particularly those noted herein or their pharmaceutically acceptable salts which are active when given orally, can be formulated as
35 liquids, for example syrups, suspensions or emulsions, tablets, capsules and lozenges.

A liquid formulation will generally consist of a suspension or solution of the compound or pharmaceutically acceptable salt in a suitable liquid carrier(s) for example, ethanol, glycerin, non-aqueous solvent, for example polyethylene glycol, oils, or water with a suspending agent, preservative, flavouring or colouring agent.

A composition in the form of a tablet can be prepared using any suitable pharmaceutical carrier(s) routinely used for preparing solid formulations. Examples of such carriers include magnesium stearate, starch, lactose, sucrose and cellulose.

5 A composition in the form of a capsule can be prepared using routine encapsulation procedures. For example, pellets containing the active ingredient can be prepared using standard carriers and then filled into a hard gelatin capsule; alternatively, a dispersion or suspension can be prepared using any suitable pharmaceutical carrier(s), for example aqueous gums, celluloses, silicates or oils and the dispersion or suspension then filled into a soft gelatin capsule. Preferably the composition is in unit
10 dose form such as a tablet or capsule.

Typical parenteral compositions consist of a solution or suspension of the compound or pharmaceutically acceptable salt in a sterile aqueous carrier or parenterally acceptable oil, for example polyethylene glycol, polyvinyl pyrrolidone, lecithin, arachis oil or sesame oil. Alternatively, the solution can be lyophilized and then reconstituted with a
15 suitable solvent just prior to administration.

A typical suppository formulation comprises a compound or a pharmaceutically acceptable salt thereof which is active when administered in this way, with a binding and/or lubricating agent such as polymeric glycals, gelatins or cocoa butter or other low melting vegetable or synthetic waxes or fats.

20 The pharmaceutically acceptable compounds of the invention will normally be administered to a subject in a daily dosage regimen. For a patient this may be, for example, from about .001 to about 100mg/kg, preferably from about 0.001 to about 10mg/kg animal body weight. A daily dose, for a larger mammal is preferably from about 1 mg to about 1000 mg, preferably between 1 mg and 500 mg or a pharmaceutically acceptable salt thereof,
25 calculated as the free base, the compound being administered 1 to 4 times per day. Unit dosage forms may contain from about 25 μ g to about 500mg of the compound.

There are many diseases and conditions in which dysregulation of apoptosis plays an important role. All of these conditions involve undesired, deleterious loss of specific
30 cells with resulting pathological consequences.

Bone remodeling involves the initial resorption by osteoclasts, followed by bone formation by osteoblasts. Recently, there have been a number of reports of apoptotic events occurring during this process. Apoptotic events have been observed in both the bone forming and bone resorbing cells *in vitro* and indeed at the sites of these remodeling units
35 *in vivo*.

Apoptosis has been suggested as one of the possible mechanisms of osteoclast disappearance from reversal sites between resorption and formation. TGF- β 1 induces apoptosis (approx. 30%) in osteoclasts of murine bone marrow cultures grown for 6 days *in vitro*. (Hughes, et al., *J. Bone Min. Res.* 9, S138 (1994)). The anti-resorptive 5 bisphosphonates (clodronate, pamidronate or residronate) promote apoptosis in mouse osteoclasts *in vitro* and *in vivo*. (Hughes, et al., supra at S347). M-CSF, which has previously been found to be essential for osteoclast formation can suppress apoptosis, suggesting not only that maintenance of osteoclast populations, but also that formation of these multinucleated cells may be determined by apoptosis events. (Fuller, et al., *J. Bone* 10 *Min. Res.* 8, S384 (1993); Perkins, et al., *J. Bone Min. Res.* 8, S390 (1993)). Local injections of IL-1 over the calvaria of mice once daily for 3 days induces intense and aggressive remodeling. (Wright, et al., *J. Bone Min. Res.* 9, S174 (1994)). In these studies, 1% of osteoclasts were apoptotic 1 day after treatment, which increased 3 days later to 10%. A high percentage (95%) of these apoptotic osteoclasts were at the reversal site. 15 This data suggests that Caspases are functionally very important in osteoclast apoptosis.

Therefore, one aspect of the present invention is the promotion of apoptosis in osteoclasts as a novel therapy for inhibiting resorption in diseases of excessive bone loss, such as osteoporosis, using compounds of Formula (I) as defined herein.

20 Apoptosis can be induced by low serum in highly differentiated rat osteoblast-like (Ros 17/2.8) cells (Ihbe, et al., (1994) *J. Bone Min. Res.* 9, S167)). This was associated with a temporal loss of osteoblast phenotype, suggesting that maintenance of lineage specific gene expression and apoptosis are physiologically linked. Fetal rat calvaria derived osteoblasts grown *in vitro* undergo apoptosis and this is localized to areas of nodule 25 formation as indicated by *in situ* end-labeling of fragmented DNA. (Lynch, et al., (1994) *J. Bone Min. Res.* 9, S352). It has been shown that the immediate early genes c-fos and c-jun are expressed prior to apoptosis; c-fos and c-jun-Lac Z transgenic mice show constitutive expression of these transcription factors in very few tissues, one of which is bone (Smeyne, et al., (1992) *Neuron*. 8, 13-23; and Morgan, J. (1993) *Apoptotic Cell* 30 Death: Functions and Mechanisms. Cold Spring Harbor 13-15th October). Apoptosis was observed in these animals in the epiphyseal growth plate and chondrogenic zones as the petula ligament calcifies. Chondrogenic apoptosis has also been observed in PTHRP-less mice and these transgenics exhibit abnormal endochondral bone formation (Lee, et al., (1994) *J. Bone Min. Res.* 9, S159). A very recent paper examined a human osteosarcoma 35 cell line which undergoes spontaneous apoptosis. Using this cell line, LAP-4, but not ICE, could be detected and *in vitro* apoptosis could be blocked by inhibition or depletion of LAP-4 (Nicholson, et al., (1995) *Nature* 376, 37-43). Thus, apoptosis may play a role in

loss of osteoblasts and chondrocytes and inhibition of apoptosis could provide a mechanism to enhance bone formation.

Therefore, another aspect of the present invention is the inhibition of apoptosis as a novel therapy to enhance bone formation using compounds of Formula (I) as defined
5 herein.

Osteoarthritis (OA) is a degenerative disease characterized by progressive erosion of articular cartilage. Chondrocytes are the single cell-type found in articular cartilage and perturbations in metabolism of these cells may be involved in the pathogenesis of OA.
Injury to cartilage initiates a specific reparative response which involves an increase in the
10 production of proteoglycan and collagen in an attempt to reestablish normal matrix homeostasis. However, with the progress of the disease, the 3-dimensional collagen network is disrupted and cell death of chondrocytes occurs in OA lesions (Malemud, et al.: Regulation of chondrocytes in osteoarthritis. In: Adolphe, M. ed. Biological Regulation of Chondrocytes. Boca Raton:CRC Press, 1992, 295-319). It has been shown that in OA,
15 chondrocytes adjacent to cartilage defects express high levels of bcl-2 (Erlacher, et al., (1995) *J. of Rheumatology*, 926-931). This represents an attempt to protect chondrocytes from apoptosis induced by the disease process.

Protection of chondrocytes during early degenerative changes in cartilage by inhibition of apoptosis may provide a novel therapeutic approach to this common disease.
20 Therefore, another aspect of the present invention is the inhibition of apoptosis as a novel therapy to treat osteoarthritis, using compounds of Formula (I) as defined herein.

Recent evidence shows that chronic, degenerative conditions of the liver are linked to hepatocellular apoptosis. These conditions include chemical-, infectious- and
25 immune/inflammatory-induced hepatocellular degeneration. Apoptosis of liver cells has been observed in liver degenerative states induced by a variety of chemical agents, including acetaminophen (Ray, et al.,(1993) *FASEB J.* 7, 453-463), cocaine (Cascales, et al., (1994) *Hepatology* 20, 992-1001) and ethanol (Baroni, et al., (1994) *J. Hepatol.* 20, 508-513). Infectious agents and their chemical components that have been shown to induce
30 apoptosis include hepatitis ((Hiramatsu, et al., (1994) *Hepatology* 19, 1354-1359; Mita, et al., (1994) *Biochem. Biophys. Res. Commun.* 204, 468-474)), tumor necrosis factor and endotoxin . (Leist, et al., (1995) *J. Immunol.* 154, 1307-1316; and Decker, K. (1993) *Gastroenterology* 28(S4), 20-25). Stimulation of immune / inflammatory responses by mechanisms such as allograft transplantation and hypoxia followed by reperfusion have
35 been shown to induce apoptosis of hepatocytes (Krams, et al., (1995) *Transplant. Proc.* 27,

466-467). Together, this evidence supports that hepatocellular apoptosis is central to degenerative liver diseases.

Therefore, another aspect of the present invention is the inhibition of apoptosis as a novel therapy to treat degenerative liver diseases., using compounds of Formula (I) as defined herein.

Apoptosis is recognized as a fundamental process within the immune system where cell death shapes the immune system and effects immune functions. Apoptosis also is implicated in viral diseases (e.g AIDS). Recent reports indicate that HIV infection may produce an excess of apoptosis, contributing to the loss of CD4⁺ T cells. Of additional interest is the observation that APO-1/Fas shares sequence homology with HIV-1 gp120.

Therefore, another aspect of the present invention is the inhibition of apoptosis as a novel therapy to treat viral diseases. using compounds of Formula (I) as defined herein.

Additional therapeutic directions and other indications in which inhibition of apoptotic cysteine proteases is of therapeutic utility, along with relevant citations in support of the involvement for apoptosis in each indication, are presented below in Table 1.

Table 1: Therapeutic Indications Related to Apoptosis

| Indication | Citations |
|----------------------------------|--|
| Ischemia / reperfusion | Barr et al., (1994) <i>BioTechnology</i> 12 , 487-493; Thompson, C. B. (1995) <i>Science</i> 267 , 1456-1462 |
| Stroke | Barr et al supra; and Thompson, C., supra |
| Polycystic kidney disease | Barr et al., supra; and Mondain, et al., (1995) <i>ORL J. Otorhinolaryngol. Relat. Spec.</i> 57 , 28-32 |
| Glomerulo-nephritis | Barr et al., supra |
| Osteoporosis | Lynch et al., (1994) <i>J. Bone Min. Res.</i> 9 , S352; Nicholson et al., (1995) <i>Nature</i> 376 , 37-43 |
| Erythropoiesis / Aplastic anemia | Thompson, C., supra; Koury et al., (1990) <i>Science</i> 248 , 378-381 |

| | |
|-------------------------------|---|
| Chronic liver degeneration | Thompson, C., <i>supra</i> ; Mountz et al., 1994) <i>Arthritis Rheum.</i> 37 , 1415-1420; Goldin et al., (1993) <i>Am. J. Pathol.</i> 171 , 73-76 |
| T-cell death | Thompson, C., <i>supra</i> ; Ameison et al., (1995) <i>Trends Cell Biol.</i> 5 , 27-32 |
| Osteoarthritis - chondrocytes | Ishizaki et al., (1994) <i>J. Cell Biol.</i> 126 , 1069-1077; Blanco et al., (1995) <i>Am. J. Pathol.</i> 146 , 75-85 |
| Male pattern baldness | Mondain et al., <i>supra</i> ; Seiberg et al., (1995) <i>J. Invest. Dermatol.</i> 104 , 78-82; Tamada et al., (1994) <i>Br. J. Dermatol.</i> 131 , 521-524 |
| Alzheimer's disease | Savill, J.,(1994) <i>Eur. J. Clin. Invest.</i> 24 , 715-723; Su et al., (1994) <i>Neuroreport</i> 5 , 2529-2533; Johnson, E., (1994) <i>Neurobiol. Aging</i> 15 Suppl. 2 , S187-S189 |
| Parkinson's disease | Savill, J., <i>supra</i> ; Thompson, C.. <i>supra</i> |
| Type I diabetes | Barr et al., <i>supra</i> |

The IL-1 and TNF inhibiting effects of compounds of the present invention are determined by the following *in vitro* assays:

Interleukin - 1 (IL-1)

- 5 Human peripheral blood monocytes are isolated and purified from either fresh blood preparations from volunteer donors, or from blood bank buffy coats, according to the procedure of Colotta *et al*, *J Immunol*, **132**, 936 (1984). These monocytes (1×10^6) are plated in 24-well plates at a concentration of 1-2 million/ml per well. The cells are allowed to adhere for 2 hours, after which time non-adherent cells are removed by gentle washing. Test compounds are then 10 added to the cells for about 1 hour before the addition of lipopolysaccharide (50 ng/ml), and the cultures are incubated at 37°C for an additional 24 hours. At the end of this period, culture super-natants are removed and clarified of cells and all debris. Culture supernatants are then immediately assayed for IL-1 biological activity, either by the method of Simon *et al.*, *J. Immunol. Methods*, **84**, 85, (1985) (based on ability of IL-1 to stimulate a Interleukin 2

producing cell line (EL-4) to secrete IL-2, in concert with A23187 ionophore) or the method of Lee *et al.*, J. ImmunoTherapy, 6 (1), 1-12 (1990) (ELISA assay).

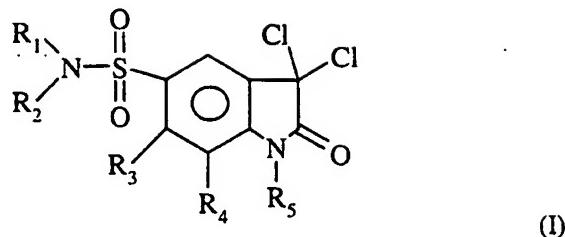
Tumour Necrosis Factor (TNF):

5 Human peripheral blood monocytes are isolated and purified from either blood bank buffy coats or platelet pheresis residues, according to the procedure of Colotta, R. *et al.*, J Immunol, 132(2), 936 (1984). The monocytes are plated at a density of 1×10^6 cells/ml medium/well in 24-well multi-dishes. The cells are allowed to adhere for 1 hour after which time the supernatant is aspirated and fresh medium (1ml, RPMI-1640, Whitaker Biomedical
10 Products, Whitaker, CA) containing 1% fetal calf serum plus penicillin and streptomycin (10 units/ml) added. The cells are incubated for 45 minutes in the presence or absence of a test compound at 1nM-10mM dose ranges (compounds are solubilized in dimethyl sulfoxide/ethanol, such that the final solvent concentration in the culture medium is 0.5% dimethyl sulfoxide/0.5% ethanol). Bacterial lipopoly-saccharide (*E. coli* 055:B5 [LPS] from
15 Sigma Chemicals Co.) is then added (100 ng/ml in 10 ml phosphate buffered saline) and cultures incubated for 16-18 hours at 37°C in a 5% CO₂ incubator. At the end of the incubation period, culture supernatants are removed from the cells, centrifuged at 3000 rpm to remove cell debris. The supernatant is then assayed for TNF activity using either a radio-immuno or an ELISA assay, as described in WO 92/10190 and by Becker *et al.*, J Immunol, 1991, 147, 4307.

20 The above description fully discloses the invention including preferred embodiments thereof. Modifications and improvements of the embodiments specifically disclosed herein are within the scope of the following claims. Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the
25 present invention to its fullest extent. Therefore the Examples herein are to be construed as merely illustrative and not a limitation of the scope of the present invention in any way. The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows.

What is claimed is:

1. A compound of formula



5

wherein

R₁ is hydrogen, or C₁₋₄ alkyl;

R₂ is C₁₋₁₀ alkyl, optionally substituted arylC₁₋₄alkyl, optionally substituted heteroaryl

C₁₋₄ alkyl, optionally substituted C₃₋₇ cycloalkyl, or R₁ and R₂ together with the

10 nitrogen to which they are attached from a 3 to 10 membered ring which optionally contains an additional heteroatom selected from oxygen, nitrogen or sulfur;

R₃ and R₄ are C₁₋₆alkyl, hydrogen, nitro, or halogen and

R₅ is C₁₋₆alkyl, hydrogen, arylalkyl or heteroarylkyl.

15 2. A compound according to Claim 1 wherein R₅ is substituted benzyl.

3. A compound according to Claim 1 wherein R₁ is hydrogen or methyl.

4. A compound wherein R₁ and R₂ are joined to form a five membered nitrogen

20 containing ring.

5. The compound according to claim 1 which is

5-Chlorosulfonyl-3,3-dichloro-2-oxindole

5-Benzylaminosulfonyl-3,3-dichloro-2-oxindole

25 5-[N-(1-Methyl-3-phenylpropylamino)sulfonyl]-3,3-dichloro-2-oxindole

5-[N-(N-Benzyl-2-cyanoethylamino)sulfonyl]-3,3-dichloro-2-oxindole

5-[N-(2-(3-Pyridyl)ethylamino)sulfonyl]-3,3-dichloro-2-oxindole

5-[N-(2-Furyl)amino)sulfonyl]-3,3-dichloro-2-oxindole

5-[N-(2-Isopropoxyethylamino)sulfonyl]-3,3-dichloro-2-oxindole

30 5-[N-(2-Methoxyethylamino)sulfonyl]-3,3-dichloro-2-oxindole

5-[N-(2-Tetrahydrofuryl)amino)sulfonyl]-3,3-dichloro-2-oxindole

(-)-5-[N-(cis-Myrtanyl)amino)sulfonyl]-3,3-dichloro-2-oxindole

5-[(1-Benzylpiperidinyl-4-amino)sulfonyl]-3,3-dichloro-2-oxindole

- 5-[N-(2-Indanamino)sulfonyl]-3,3-dichloro-2-oxindole
5-[N-(Cyclopropylmethylamino)sulfonyl]-3,3-dichloro-2-oxindole
5-[N-(1,5-Dimethylhexylamino)sulfonyl]-3,3-dichloro-2-oxindole
5-[N-(N-Methylbenzylamino)sulfonyl]-3,3-dichloro-2-oxindole
5 (+/-)-5-[N-(3-(N-Acetyl-N-methylamino)pyrrolidinyl)sulfonyl]-3,3-dichloro-2-oxindole
5-[2-(1,2,3,4-Tetrahydroisoquinolino)sulfonyl]-3,3-dichloro-2-oxindole
(+/-)-5-[1-(Decahydroisoquinolino)sulfonyl]-3,3-dichloro-2-oxindole
5-[N-(N-Methyl-2-cyanoethylamino)sulfonyl]-3,3-dichloro-2-oxindole
5-[N-(N-Methylcyanomethylamino)sulfonyl]-3,3-dichloro-2-oxindole
10 5-[N-(Pyrrolidinyl)sulfonyl]-3,3-dichloro-2-oxindole
5-[N-(N-Methylphenethylamino)sulfonyl]-3,3-dichloro-2-oxindole
5-[N-(Azacyclooctane)sulfonyl]-3,3-dichloro-2-oxindole
5-[N-(3-Azabicyclo[3.2.2]nonane)sulfonyl]-3,3-dichloro-2-oxindole
5-[1-(2-Ethoxycarbonylpiperidinyl)sulfonyl]-3,3-dichloro-2-oxindole
15 5-[N-(Morpholino)sulfonyl]-3,3-dichloro-2-oxindole
(S)-(+)-5-[1-(2-Methoxymethylpyrrolidinyl)sulfonyl]-3,3-dichloro-2-oxindole
5-[N-(N-Methyl-2-(4-pyridinyl)ethylamino)sulfonyl]-3,3-dichloro-2-oxindole
5-[N-(N-Methyl-2-hydroxyethylamino)sulfonyl]-3,3-dichloro-2-oxindole
(S)-(+)-5-[N-(2-Hydroxymethylpyrrolidinyl)sulfonyl]-3,3-dichloro-2-oxindole
20 5 (+/-)-5-[1-(3-Hydroxypyrrolidinyl)sulfonyl]-3,3-dichloro-2-oxindole
(+/-)-5-[1-(3-aminocarbonylpiperidinyl)sulfonyl]-3,3-dichloro-2-oxindole
(+/-)-5-[1-(2-Methylpiperidinyl)sulfonyl]-3,3-dichloro-2-oxindole
(+/-)-5-[1-(4-Methylpiperidinyl)sulfonyl]-3,3-dichloro-2-oxindole
5-[1-(4-Hydroxypiperidinyl)sulfonyl]-3,3-dichloro-2-oxindole
25 5 (+/-)-5-[1-(2-(2-Hydroxyethyl)piperidinyl)sulfonyl]-3,3-dichloro-2-oxindole
(+/-)-5-[1-(3-Hydroxymethylpiperidinyl)sulfonyl]-3,3-dichloro-2-oxindole
5-[1-(4-Phenylpiperidinyl)sulfonyl]-3,3-dichloro-2-oxindole
5-[1-(4-Benzylpiperidinyl)sulfonyl]-3,3-dichloro-2-oxindole
5-[1-(4-(1-Piperidinyl)piperidinyl)sulfonyl]-3,3-dichloro-2-oxindole
30 5-Benzylaminosulfonyl-N-methyl-3,3-dichloro-2-oxindole
5-Chlorosulfonyl-N-methyl-3,3-dichloro-2-oxindole
5-Benzylaminosulfonyl-N-methyl-3,3-dichloro-2-oxindole
N-Methyl-5-(1-piperidinylsulfonyl)-3,3-dichloro-2-oxindole
35 6. A pharmaceutical composition comprising a compound according to Claim 1 and a pharmaceutically acceptable carrier or diluent.

7. A method of blocking excess or inappropriate apoptosis in a mammal in need of such treatment which method comprises administering to said mammal or human an effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

5

8. The method according to Claim 7 wherein the excessive or inappropriate apoptosis occurs in Alzheimer disease.

9. The method according to Claim 7 wherein the excessive or inappropriate 10 apoptosis occurs in viral infections.

10. The method according to Claim 7 wherein the excessive or inappropriate apoptosis occurs during infarction or reperfusion injury.

15 11. The method according to Claim 7 wherein the excessive or inappropriate apoptosis occurs during ischemia.

12. The method according to Claim 7 wherein the excessive or inappropriate results in excessive bone loss.

20

13. The method according to Claim 7 wherein the excessive or inappropriate apoptosis results in the disease of osteoarthritis.

25 14. The method according to Claim 7 wherein the excessive or inappropriate apoptosis results in hepatocellular degeneration.

15. A method for the treatment of diseases or disorders associated with excessive IL-1 β convertase activity, in a mammal in need thereof, which method comprises administering to said mammal an effective amount of a compound of Formula (I), or a 30 pharmaceutically acceptable salt thereof.

16. A method of blocking or decreasing the production of IL-1 β and/or TNF, in a mammal in need of such treatment, which method comprises administering to said mammal an effective amount of a compound of Formula (I), or a pharmaceutically 35 acceptable salt thereof.

17. A method for inhibiting the production of caspase three and seven in a mammal in need of such treatment, which method comprises administering to said mammal an effective amount of a compound of Formula I, or a pharmaceutically acceptable salt thereof.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US98/15909

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) A61K 31/40

US CL 514/490

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/490

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|-----------|---|-----------------------|
| A | Database CAPLUS on STN, AN 1997:240726, LEVY et al, "Preparation of Cephem Oxides as inhibitors of interleukin converting enzyme and apoptosis", WO 9708174 A1, 03 June 1997. | 1-17 |
| A,E | US 5,763,220 A (HILLMAN et al) 06 September 1998, Col. 1, lines 10-45. | 1-17 |

| | | | |
|---|--|--------------------------|--|
| <input type="checkbox"/> | Further documents are listed in the continuation of Box C. | <input type="checkbox"/> | See patent family annex. |
| * Special categories of cited documents: | | "T" | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| "A" document defining the general state of the art which is not considered to be of particular relevance | | "X" | document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| "B" earlier document published on or after the international filing date | | "Y" | document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | | "A" | document member of the same patent family |
| "O" document referring to an oral disclosure, use, exhibition or other means | | | |
| "P" document published prior to the international filing date but later than the priority date claimed | | | |

| | |
|---|---|
| Date of the actual completion of the international search | Date of mailing of the international search report |
| 30 SEPTEMBER 1998 | 22 OCT 1998 |
| Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 | Authorized officer <i>J. Lawrence Jr.</i> KEITH MACMILLAN |
| Facsimile No. (703) 305-3230 | Telephone No. (703) 308-1235 |

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- BLACK BORDERS**
- IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- FADED TEXT OR DRAWING**
- BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- SKEWED/SLANTED IMAGES**
- COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- GRAY SCALE DOCUMENTS**
- LINES OR MARKS ON ORIGINAL DOCUMENT**
- REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- OTHER: _____**

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.